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Has received an application for a patent for a new and useful invention. The title and description of the invention are enclosed. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law.

Therefore, this

United States Patent

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Michelle K. Lee

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(12) **United States Patent**
Peneva et al.

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(54) **PROBIOTICS FOR DIETARY DAIRY PRODUCT**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 212 days.

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(52) **U.S. Cl.**

CPC ***A23C 9/127*** (2013.01); ***A23L 1/3014*** (2013.01); ***C12N 1/20*** (2013.01); ***C12R 1/225*** (2013.01); ***C12R 1/46*** (2013.01); *A23Y 2220/29* (2013.01); *A23Y 2220/39* (2013.01); *A23Y 2240/75* (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

(56) **References Cited**

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(57) **ABSTRACT**

The present invention relates to compositions containing probiotic microorganisms and methods of making and using probiotic associations containing these microorganisms. More particularly, the invention relates to an association of probiotic lactic acid microorganisms including newly identified strains of microorganisms and their use in dietary products. These microorganisms are cultured to produce a protective shell that enhances shelf-stability of compositions and products of the invention. The isolated microorganisms include new strains of *Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus helveticus, Lactobacillus delbrueckii* subsp. *lactis*, and *Streptococcus thermophillus.*

11 Claims, 16 Drawing Sheets

TABLE 8-continued

INDICATORS	ANALYSIS METHODS	CHARACTERISTICS AND NORMS	RESULTS
Total number of mesophilic aerobic and facutative anaerobic non-lactic acid bacteria, CFU/g, not more than:	BDS ISO 6610	1000 ($1.0 \cdot 10^3$)	Meets the requirements
Coliforms in 1.0 g of the product:	BDS ISO 4831	To be established	Meets the requirements
Yeast and mold in 1.0 g of the product:	BDS ISO 6611	To be established	Meets the requirements
Microscopic mold fungi spores B 1.0 g of the product:	BDS ISO 6611	To be established	Meets the requirements
Salmonella species in 0.25 g of the product:	BDS ISO 6579	To be established	Meets the requirements
Coagulasopositive *staphylococci* (*S. aureus*) in 1.0 g of the product:	BDS ISO 6888-1, 2, 3	To be established	Meets the requirements
Listeria monocytogenes in 25.0 g of the product:	BDS ISO 11290-1	To be established	Meets the requirements
Aflatoxin M1 mg/kg	BDS 16242-85	No more than 0.5	Meets the requirements

Example 8

Food Supplements for Healthy Human Nutrition Added to Coffee

In order to obtain food supplements for healthy human nutrition added to coffee, 4 g of dietary milk product was obtained as described in Example 3. Then, 2 g of instant coffee or decaf coffee was added, in addition to 2 g of creamer and 2 g of dextrose monohydrate. The mixture was homogenized and wrapped in aluminium foil. The resulting product has a refreshing and healthy effect when consumed.

Example 9

Food Supplement for Healthy Human Nutrition Containing Blueberry Extract

In order to manufacture capsules of a dietary product containing blueberry extract, 240 mg of product obtained as described in Example 5 was added to 10 mg of dry blueberry extract, standardized to 25% contents of Anthocyanins and 20 mg dextrose monohydrate. The obtained mixture was homogenized and placed in a plant capsule. The product was used as a food supplement for maintaining the overall health and eyesight, in particular.

Example 10

Food Supplements Containing Papaya Extract

In order to obtain capsules of a dietary product containing papaya extract, 250 mg of product obtained as described in Example 5 were added to 15 mg of dry papaya extract and 20 mg of dextrose monohydrate. The obtained mixture was homogenized and placed in a gelatin capsule. The product was used for maintaining and optimising the body weight.

The invention illustratively disclosed herein suitably may be practiced in the absence of any element, which is not specifically disclosed herein. It is apparent to those skilled in the art, however, that many changes, variations, modifications, other uses, and applications to the method are possible, and also changes, variations, modifications, other uses, and applications which do not depart from the spirit and scope of the invention are deemed to be covered by the invention, which is limited only by the claims which follow.

What is claimed is:

1. A method of producing a dietary product comprising:

a) mixing spring water from Mountain Stara Planina in Northern Bulgaria with pasteurized milk in a ratio of about 1:2 (v/v) to allow microorganisms in the spring water to multiply and to produce a starter material wherein the microorganisms in the spring water comprise strains: *Lactobacillus delbruecki* subsp. *bulgaricus* DWT1 registered in CCM under no. 7992, *Lactobacillus helveticus* DWT2 registered in CCM under no. 7993, *Lactobacillus delbrueckii* subsp. *lactis* DWT3 registered in CCM under no. 7994, *Streptococcus thermophilus* DWT4 registered in CCM under no. 7992, *Streptococcus thermophilus* DWT5 registered in CCM under no. 7993, *Streptococcus thermophilus* DWT6 registered in CCM under no. 7994, *Streptococcus thermophilus* DWT7 registered in CCM under no. 7995 and *Streptococcus thermophilus* DWT8 registered in CCM under no. 7996;

b) mixing the starter material of step a) with pasteurized milk in a ratio of about 3:10 (g/L) to allow fermentation; and,

c) lyophilizing the fermentation product to produce a dietary product.

2. The method of claim **1** further comprising combining the dietary product with a nutritional supplement.

3. The method of claim **1**, wherein the dietary product is shelf stable for at least 12 months.

4. The method of claim **1**, wherein the dietary product is shelf stable for at least 22 months.

5. A method of producing a starter material, the method comprising mixing spring water from Mountain Stara Planina in Northern Bulgaria with pasteurized milk in a ratio of about 1:2 (v/v) to allow microorganisms in the spring water to

multiply and to produce a biomass for use as a starter material starter material; wherein the microorganisms in the spring water comprise strains: *Lactobacillus delbrueckii* subsp. *bulgaricus* DWT1 registered in CCM under no. 7992, *Lactobacillus helveticus* DWT2 registered in CCM under no. 7993, *Lactobacillus delbrueckii* subsp. *lactis* DWT3 registered in CCM under no. 7994, *Streptococcus thermophilus* DWT4 registered in CCM under no. 7992, *Streptococcus thermophilus* DWT5 registered in CCM under no. 7993, *Streptococcus thermophilus* DWT6 registered in CCM under no. 7994, *Streptococcus thermophilus* DWT7 registered in CCM under no. 7995 and *Streptococcus thermophilus* DWT8 registered in CCM under no. 7996.

6. A method of producing a dietary product comprising:

a) mixing a starter material obtained from using the method of claim **5** with pasteurized milk in a ratio of about 3:10 (g/L) to allow fermentation; and

b) lyophilizing the fermentation product of step a) to produce a dietary product.

7. The method of claim **6**, wherein the dietary product after lyophilizing contains alive cells of probiotic association with concentration from 8.0×10^7 cfu/g to 1.2×10^8 cfu/g,

wherein the concentration of alive cells of the strains *Streptococcus thermophilus* ranges from 4.6×10^7 cfu/g to 5.9×10^7 cfu/g, including the strain *Streptococcus thermophilus* DWT4, CCM reg. No. 7992, strain *Streptococcus thermophilus* DWT5, CCM reg. No. 7993, strain *Streptococcus thermophilus* DWT6, CCM reg. No. 7994, strain *Streptococcus thermophilus* DWT7, CCM reg. No. 7995 and strain *Streptococcus thermophilus* DWT8, CCM reg. No. 7996;

wherein the concentration of alive cells of strain *Lactobacillus delbrueckii* subsp. *lactis*, CCM reg. No. 7994 ranges from 6.1×10^6 cfu/g to 7.8×10^6 cfu/g,

wherein the concentration of alive cells of strain *Lactobacillus delbruecki* subsp. *bulgaricus*, CCM reg. No. 7992 ranges from 1.4×10^7 cfu/g to 2.9×10^7 cfu/g and the concentration of alive cells of the strain *Lactobacillus helveticus*, CCM reg. No. 7993 ranges from 1.4×10^7 to 2.8×10^7 cfu/g.

8. The method of claim **6**, wherein the dietary product after rehydration and in the extreme conditions of the gastro-intestinal tract contains a concentration no less than 1.1×10^7 cfu/g of alive cells of the strain selected from *Lactobacillus delbrueckii* subsp. *bulgaricus DWT*1 registered in CCM under no. 7992, *Lactobacillus helveticus* DWT2 registered in CCM under no. 7993, *Lactobacillus delbrueckii* subsp. *lactis* DWT3 registered in CCM under no. 7994, *Streptococcus thermophilus* DWT4 registered in CCM under no. 7992, *Streptococcus thermophilus* DWT5 registered in CCM under no. 7993, *Streptococcus thermophilus* DWT6 registered in CCM under no. 7994, *Streptococcus thermophilus* DWT7 registered in CCM under no. 7995, and *Streptococcus thermophilus* DWT8 registered in CCM under no. 7996.

9. A method of producing a dry starter culture comprising:

a) mixing about 3 gram of a starter material obtained from using the method of claim **5** to every 10 litres of pasteurized milk to allow fermentation and produce a liquid culture;

b) mixing the liquid culture from step a) with pasteurized milk in a ratio of about 1:60 (v/v) to make a mixture and to allow fermentation;

c) neutralizing the mixture of step b), if necessary, to optimize pH for the fermentation; and

d) lyophilizing the fermentation product of step b) or c) to produce the dry starter culture.

10. A method of producing a dietary product comprising:

a) mixing a dry starter culture obtained from using the method of claim **9** with pasteurized milk in a ratio of about 3:5 (g/L) to allow fermentation; and

b) lyophilizing the fermentation product of step a) to produce a dietary product.

11. The method of claim **2**, wherein the nutritional supplement in the dietary product is selected from the group consisting of coffee, cocoa, blueberry extract, papaya extract, oils, honey, dry plant extracts, fibers, enzymes, and any combinations thereof.

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